abcam

Measuring metabolism

Simple but sophisticated assays for your plate reader, microscope, and flow cytometer

Metabolism is a complex process that lies at the core of biology. Changes to metabolism are involved in a huge range of outcomes, from cancer to neurodegeneration, and more.

Make your metabolism research less complex, with easy-to-use assay kits. Analyze live cells, lysates, and biofluids, with readout on your plate reader, microscope, or flow cytometer.

Examine:
- sugars, lipids, amino acids, the enzymes of glycolysis and the citric acid cycle, and more
- oxygen consumption, lactate production, and ATP, NADH, and similar molecules
- mitochondria and mitochondrial function
- oxidative stress, ROS, antioxidants, and related cell damage

Sugars, lipids, the enzymes of glycolysis, the citric acid cycle, and more

We offer assay kits for a large number of metabolites and metabolic enzymes, including those involved in the areas below. These assays are typically designed to be analyzed using a microplate reader.

Lipid metabolism

Sugar metabolism

Amino acid metabolism

Coenzymes and cofactors

Intermediary metabolism (including glycolysis and the citric acid cycle)

Alcohol metabolism

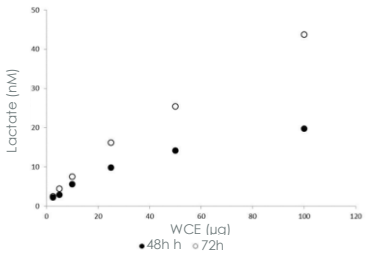

Figure 1. Lactate assay kit ab65331. HCT116 cells were cultivated for 48 and 72 hrs and sonicated in assay buffer. Image from Abreview by Mr. Christian Marx.

"The assay kit [ab65331] is a fast and easy way to quantify lactate concentrations."
- Mr Christian Marx

Oxygen consumption, lactate production, ATP, NADH, and more

Our fluorescent dye-based, high-throughput assays allow you measure metabolic throughput on your microplate reader, with no need for specialist instrumentation. Assay reactions are fully reversible, allowing measurement of time courses.

We also have conventional enzymatic lactate assays.

Assay	Readout	Notes	Assay kits
Extracellular oxygen consumption	Plate reader	Dye signal increases as cell respiration lowers O2 concentration. Oil layer isolates cells from air.	ab197243
Intracellular oxygen levels		Dye diffuses into cell. Dye fluorescence is quenched by intracellular oxygen.	ab197245
Glycolysis activity (extracellular acidification)		Lactate production during glycolysis causes acidification of the extracellular medium. Dye fluorescence increases with acidification. Kits ab222942 and ab222946 block critical steps in glycolysis and reveal disturbances to glycolysis not evident under basal conditions.	ab197244 ab222942 ab222946
Fatty acid oxidation (FAO)		Measure basal and maximum FAO-driven energy generation and delineate oxidation of exogenous, endogenous and non-long chain fatty acids.	ab217602 ab222944
Lactate		Enzymatic lactate assays	ab65330 ab65331 ab169557

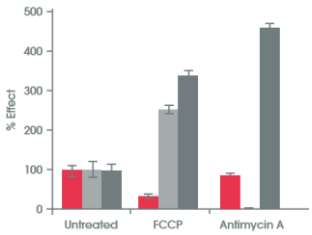

Figure 2. HepG2 cells treated with antimycin A and FCCP and tested with assays for ATP (gray, ab113849), oxygen consumption (white, ab197243) and glycolysis (black, ab197244).

ATP and ADP

ATP assays are either based on the extraction of ATP from cells followed by light production with ATP-dependent luciferase, or the ATP-dependent phosphorylation of glycerol (or other substrates) to generate a detectable product.

Assay	Readout	Notes	Assay kits
ATP	Plate reader	No-wash assay.	ab113849
ADP/ATP		No-wash assay. Same method as ATP assay. After ATP analysis, ADP is converted to ATP and detected.	ab65313
ATP		Used with cell lysates. Not as sensitive as luminescence assays.	ab83355
Phosphate			ab65622, ab219938 ab102508
Pyrophosphate			ab112155 ab179836

"We have used [ab653131] extensively to measure changes in the ATP/ADP ratio in response to changes in glucose availability and during stress tests (H2O2 treatment). Reliable kit and easy to use."
- Dr. Craig Beall

"I personally recommend ab113849] to measure extracellular ATP levels for its ease of use and for the kindly attention and help I received from the Abcam scientific support specialists."
- Dr. Lisa Sevenich

NADH, NAD, NADHP and NADP

Assay	Readout	Assay kits
NADH	Plate reader	ab186030
NAD/NADH		ab65348, ab176723, ab186032
NADP / NADPH		ab186033, ab65349, ab176724
NADPH		ab186031

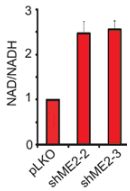

Figure 3. Ren et al. (PLoS ONE 5:e12520) used NAD/NADH assay kit (ab65348) to confirm that depletion of Malic enzyme 2, a mitochondrial enzyme involved in malate and pyruvate metabolism, increases the NAD/NADH ratio.

Mitochondrial assays

Power your mitochondrial research with assays developed by mitochondria science specialists. Abcam has been developing assays for mitochondrial research since 2004.

For more information about different methods used to analyze mitochondria, see our mitochondrial toxicity application guide.

Mitochondrial biogenesis

Measurement of mitochondrial biogenesis is becoming a standard part of drug safety characterization. Our assays measure biogenesis by measuring the ratio of mitochondrial and nuclear-encoded proteins.

Assay	Assay kits
In-Cell ELISA with fluorescence, colorimetric or fluorometric readout	ab110217, ab110216, ab140359
Western blot antibody cocktail	ab123545
ICC antibody cocktail	ab170194
Flow cytometry antibody cocktail	ab168540

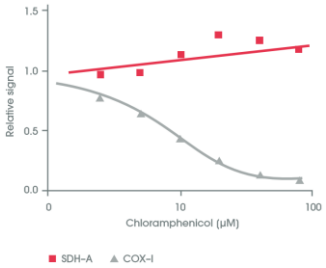

Figure 4. Inhibition of mitochondrial biogenesis by chloramphenicol, assessed using In-Cell ELISA Kit ab110217, by monitoring the relative amounts of COX-I (mitochondrial DNA encoded) and SDH-A (nuclear DNA encoded).

"I am using this kit [ab110217] for high throughput screening of more than 500 compounds. The kit is highly reproducible and I did not observe any lot to lot variation. I would highly recommend this kit."
– Dr Analeeb Sajid

Mitochondrial viability

The mitochondrial membrane potential is a key indicator of mitochondria activity levels. A number of fluorescent dyes are available that accumulate in mitochondria due to the membrane potential.

Assay	Readout	Notes	Assay kits
TMRE/TMRM	Plate reader, microscope, flow cytometer	Most popular Abcam mitochondrial membrane dye assay. Ex/Em 549/575 nm. Washed out after fixation.	ab113852
JC-1/JC-10		JC-1 (Ex/Em 530/530–570) and JC-10 (Ex/Em 590/520–570) form red aggregates at high concentrations (unaggregated dye is green). Loss of membrane potential causes loss of dye and increased green fluorescence. Washed out after fixation.	JC-1: ab113850 JC-10 (more soluble than JC-1): ab112134 ab112133
MitoNIR	Plate reader, flow cytometer	Ex/Em 635/660.	ab112149 ab11250
MitoOrange		Ex/Em 540/590.	ab138898 ab138899

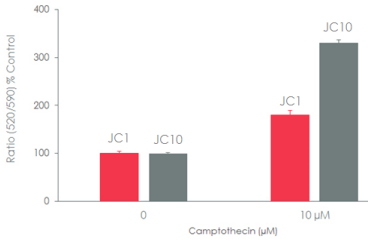

Figure 5. Mitochondrial membrane potential changes monitored with JC-10 (ab112134, red) and JC-1 (ab113850, blue).

OXPHOS enzyme complex activity assays

To investigate the in vitro activity of individual oxidative phosphorylation (OXPHOS) enzyme complexes, we use immunocapture to enable specific biochemical assays for each complex.

	Immunocapture activity assay	Complex immunocapture	Toxicity assay
Notes	Capture complex and assay activity	Enable user to develop own assays based on captured complex	Immunocapture purified beef mitochondria for in vitro treatment and assays
Complex I	ab109721	ab109711	ab109903
Complex II	ab109908	ab109799	ab109904
Complex III		ab109800	ab109905 (II + III)
Complex IV	ab109911, ab109909, ab109910	ab109801, ab109860	ab109906
Complex V			ab109907
Complete panel			ab110419

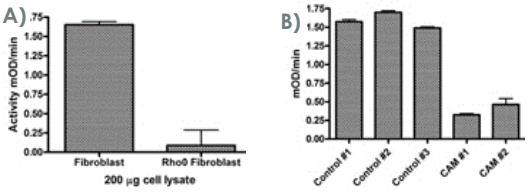

Figure 6. A) Assay kit ab109721 used to test complex I activity in normal and rho0 (complex I knockdown) fibroblasts. B) Cardiomyocytes grown in ± chloramphenicol to inhibit mitochondrial protein synthesis, Complex I activity was greatly reduced.

OXPHOS protein expression analysis

We have developed antibody cocktails for use in western blotting to enable analysis of all 5 oxphos protein complexes in a single experiment.

Assay	Assay kits
Complex I-V antibody cocktail	ab110413, ab110411, ab110412
Complex II antibody cocktail	ab110410

Apoptosis

Mitochondria are closely involved in apoptosis and cell death. For an in depth look at apoptosis see our Apoptosis Application Guide or to review available assays, tools and reagents, see our Cell Health Assays Guide.

Oxidative stress and related types of cell damage

Oxidative stress reflects the toxic side of oxygen and metabolism.

We have developed effective tools to measure reactive oxygen species (ROS) production by direct ROS measurement, quantification of ROS-induced protein modifications, and measurement of antioxidant capacity.

Direct ROS quantification

Assay	Readout	Assay kits
DCFDA – cellular reactive oxygen species	Flow cytometry, plate reader	ab113851
Cellular superoxides	Microscope, flow cytometry	ab139477
Cellular ROS/Superoxide		ab139476
Cellular reactive oxygen species	Plate reader	ab186027, ab186028, ab186029
Cellular ROS/RNS	Microscope	ab139473
Mitochondrial hydroxyl radical	Microscope, plate reader	ab219931
Mitochondrial superoxide		ab219943
Hydrogen peroxide		ab138874, ab138886, ab102500

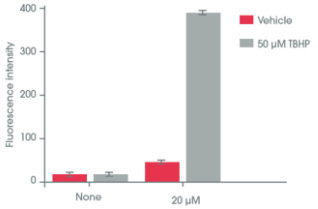

Figure 7. Detection of cellular reaction oxygen species with DCFDA assay kit ab113851. Jurkat cells were labeled with 20 µM DCFDA or unlabeled, and then cultured for 3 h in presence or absence of 50 µM tert-butyl hydrogen peroxide (TBHP).

We are currently using the product [ab113851] to measure microglial activation after 24 hours in response to activating stimuli. The product has been giving us very consistent results and is very easy to use."
- Dr Neal Bennett

Detection of cell damage

Assay	Readout	Assay kits
Lipid hydroperoxide (LPO)	Plate reader	ab133085
Lipid peroxidation (MDA)		ab118970
Protein carbonyl content		ab126287
DNA damage – apurinic/apyrimidinic sites		ab211154
Oxidized proteins	Western blot	ab178020

Quantification of antioxidant molecules

Assay	Readout	Assay kits
Total antioxidant capacity	Plate reader	ab65329
Ascorbic acid		ab65656
NAD/NADH		ab65348, ab176723
NADP/NADPH		ab65349, ab176724
GSH/GSSG ratio		ab138881, ab205811
Thiol		ab112158, ab219272
Intracellular glutathione (GSH)	Flow cytometry, plate reader	ab112132

Antioxidant enzyme capacity activity assays

Activity Assay	Readout	Assay kits
GST	Plate reader	ab65325, ab65326
Superoxide dismutase		ab65354
Glutathione reductase		ab83461
Xanthine oxidase		ab102522
Glutathione peroxidase		ab102530
Aconitase		ab109712
Catalase		ab118184, ab83464
Thioredoxin reductase		ab190804, ab83462
NQO1		ab184867
Peroxidase		ab155895
Oxidative stress defense cocktail(catalase, SOD1, TRX, smActin)	Western blot	ab179843